

# ***BlebQuant*: A MATLAB-based program for dendritic blebbing analysis**

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## **1. Background**

Ischemia induces a ‘blebbing’ of dendrites, a structural alteration where dendrites take on a ‘beads on a string’ appearance. We developed a toolkit program, *BlebQuant*, for quantitative automated bleb analysis to chart the morphology of dendrites labeled with GFP/YFP under normal conditions and after ischemia-induced damage. We have tested the toolkit using our own *in vivo* 2-photon (2P) images and found the automated results to correlate well with manual analysis and for images of different bit depths (8 to 16 bit images).

The paper entitled “Automated and quantitative image analysis of ischemic dendritic blebbing using *in vivo* 2-photon microscopy data” (Chen et al, 2011) introduced the algorithm and evaluated the accuracy of *BlebQuant*. 2P images used as examples in our paper and the software program are available for download on our website: [http://www.neuroscience.ubc.ca/faculty/murphy\\_software.html#BlebQuant](http://www.neuroscience.ubc.ca/faculty/murphy_software.html#BlebQuant).

New users of the toolkit may find it helpful to download the 2P images and follow the instructions provided in this guide. Users may find that modifications to current parameters in the algorithm may be needed for analysis on other datasets. If you encounter bugs or have technical problems with the program, please contact Shangbin Chen ([sbchen@interchange.ubc.ca](mailto:sbchen@interchange.ubc.ca)).

The MATLAB software environment (the MathWorks, Natick, MA) is required for running *BlebQuant*. Three MATLAB files (*BlebQuant.m*, *BlebQuant.fig*, *dftreregistration.m*) form *BlebQuant*. The *dftreregistration.m* used for translational registration of images was initially developed by Guizar-Sicairos (Guizar-Sicairos, 2008).

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## 2. User Guide

### Step 1. Calling *BlebQuant*

All three MATLAB files need to be placed in one folder, and the current MATLAB directory also has to match the pathway for this folder (Fig. 1). Calling “BlebQuant” on the command line (Fig. 1) will bring up the BlebQuant graphical user interface (GUI) (Fig. 2).

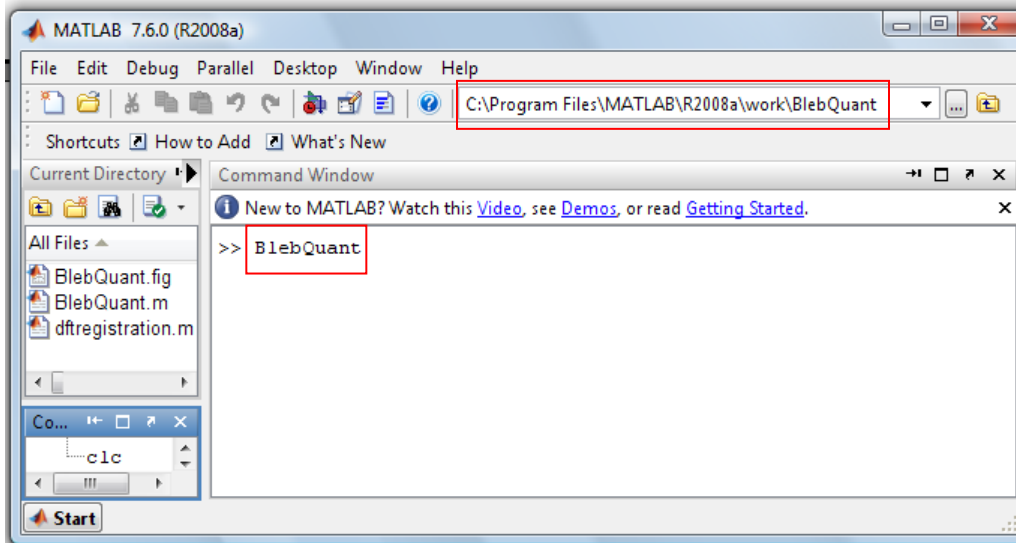


Fig. 1 Calling *BlebQuant* in MATLAB.

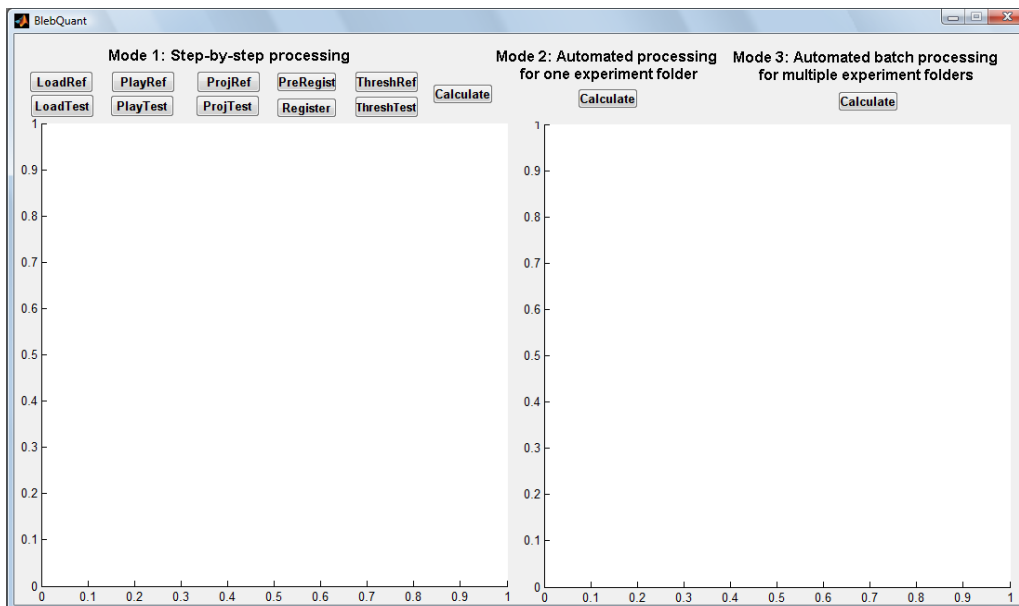


Fig. 2 *BlebQuant* graphical user interface (GUI).

## Step 2. Executing Mode 1 in *BlebQuant*

The *BlebQuant* program works under 3 different modes and in all cases the user is required to select 2P-acquired 3D image stacks for automated analysis. Mode 1 allows the user to perform step-by-step image processing and analysis for paired reference (pre stroke) and test (post stroke) image stacks. As seen in Fig. 2, the following are operations which the user selects for performing analysis: ‘LoadRef’/‘LoadTest’ for loading images, ‘PlayRef’/‘PlayTest’ for viewing 3D image stacks, ‘ProjRef’/‘ProjTest’ to z-project 3D image stacks, ‘PreRegist’ to view alignment of images, ‘Register’ for aligning images, ‘ThreshRef’/‘ThreshTest’ to convert from the grayscale (8-16 bit) to binary image, and ‘Calculate’ to determine bleb morphology values and bleb percentage for a selected image stack. Please note that “PlayRef”, “PlayTest”, “PreRegist” and “Register” are optional operations. Results can be obtained with loading reference and test image stacks, z-projecting, thresholding, and selecting “Calculate”.

In using Mode 1 of the toolkit, the user must first select a reference image and then the test image. To load the reference image file, click on “LoadRef”. A window will appear in which the user will select the reference image file (e.g. pre\_ch1.TIF). Click “Open” and the stack will load on the left-hand side of the *BlebQuant* GUI window (only the first frame of the stack will be shown). Once the file is loaded, the user may select “PlayRef” to play the selected image stack as a movie. “ProjRef” will perform a maximal intensity z-projection (Fig. 5). Loading the test image stack will execute these operations in the right window (Fig. 6).

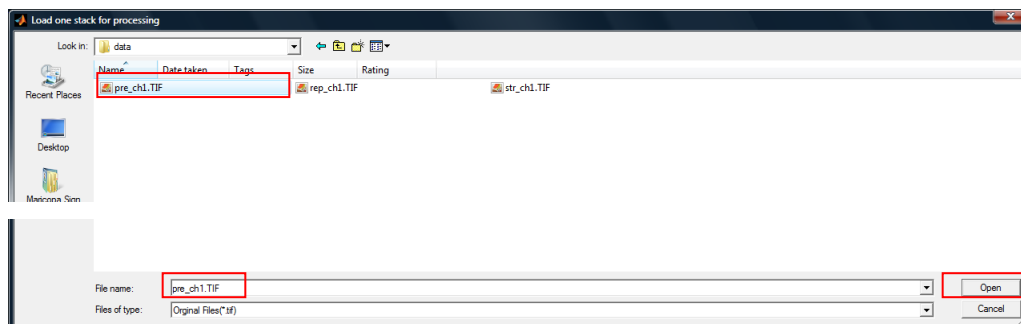


Fig. 3 Selecting a reference image stack for processing in *BlebQuant*.

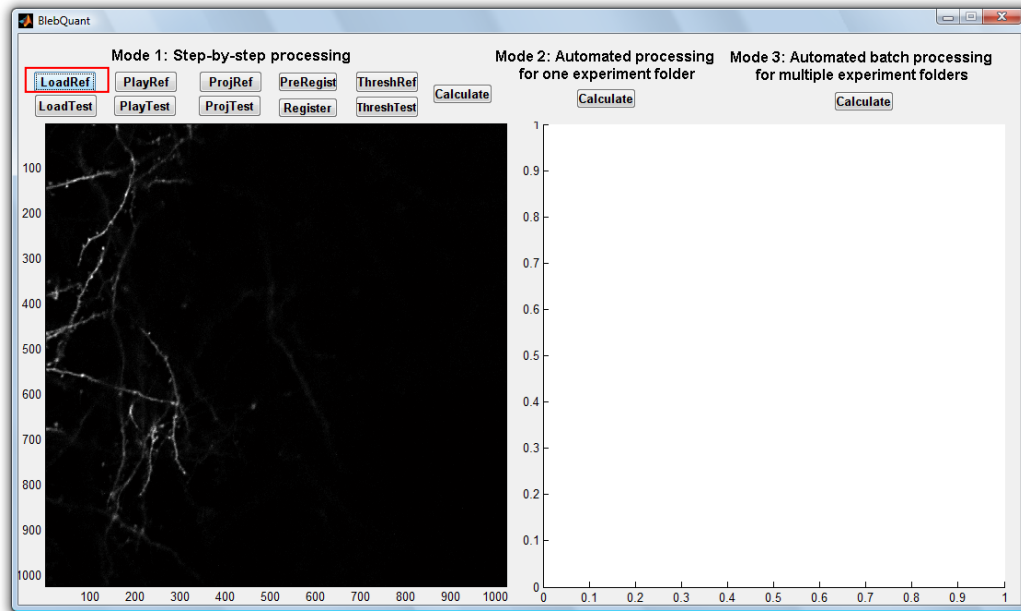


Fig. 4 Loaded reference image stack.

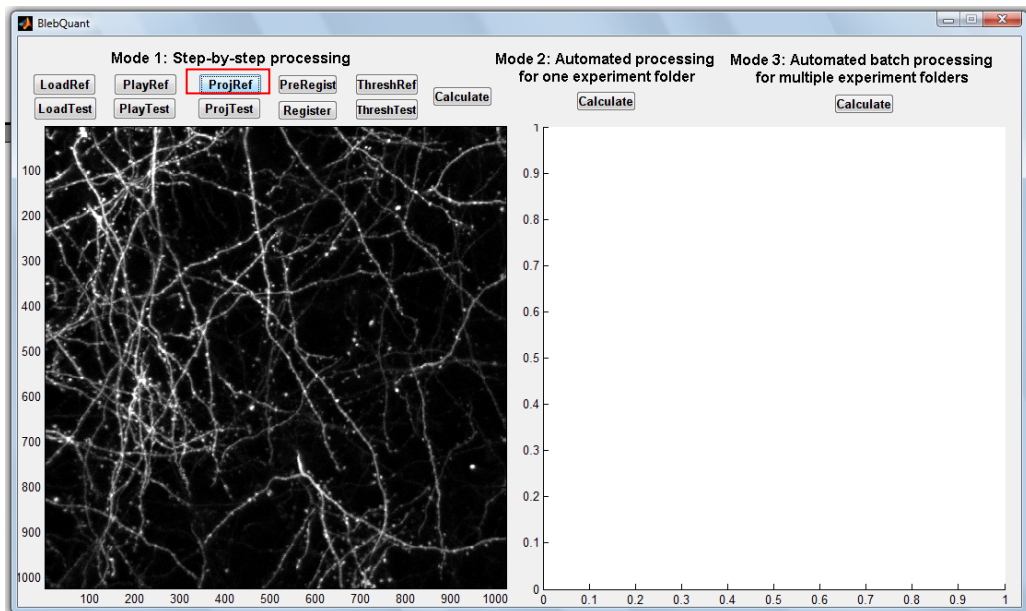


Fig. 5 Maximal intensity z-projection of reference image stack.

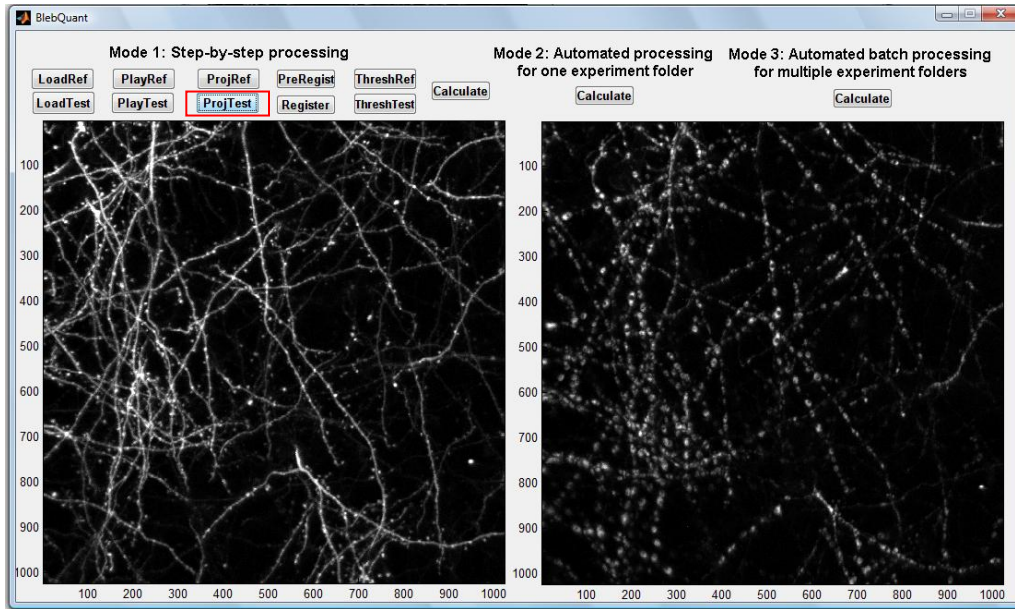


Fig. 6 Maximal intensity z-projections of reference (left) and test (right) image files.

After performing maximal intensity z-projections for both image stacks, “PreRegist” will show any relative translational movement from the projected images in a new window (Fig. 7). In this merged image, the green image will be from the reference image and the red will be from the test image. Select “Register” to align the two images with the results shown in a new window (Fig. 8).

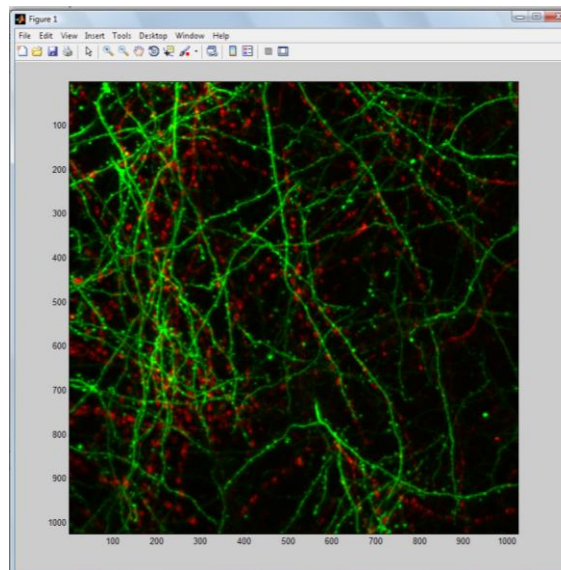


Fig. 7 Merged unregistered reference and test images.

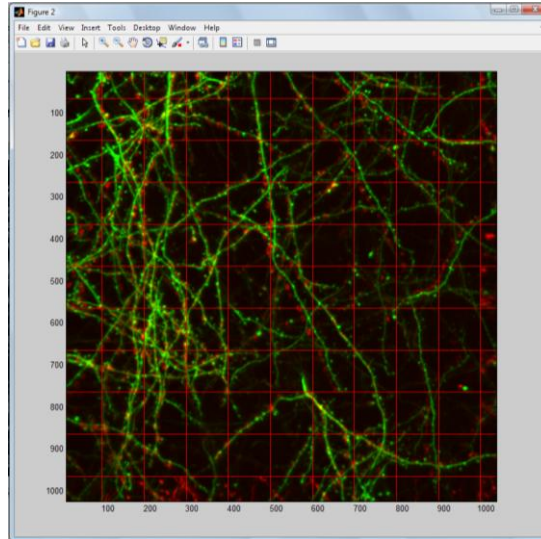


Fig. 8 Merged registered reference and test images.

One of the final operations for Mode 1 automated blebbing analysis involves image thresholding. Selecting “ThreshRef” and “ThreshTest” will convert projected images to binary images (Fig. 9). Once these operations are completed, selecting “Calculate” will execute blebbing analysis for the test image, which can be seen in the MATLAB command window. All other results are written to an excel file (Fig. 10). In Mode 1, the source code for the excel name and pathway can be found in line 549 of BlebQuant.m and be changed to the user's specifications.

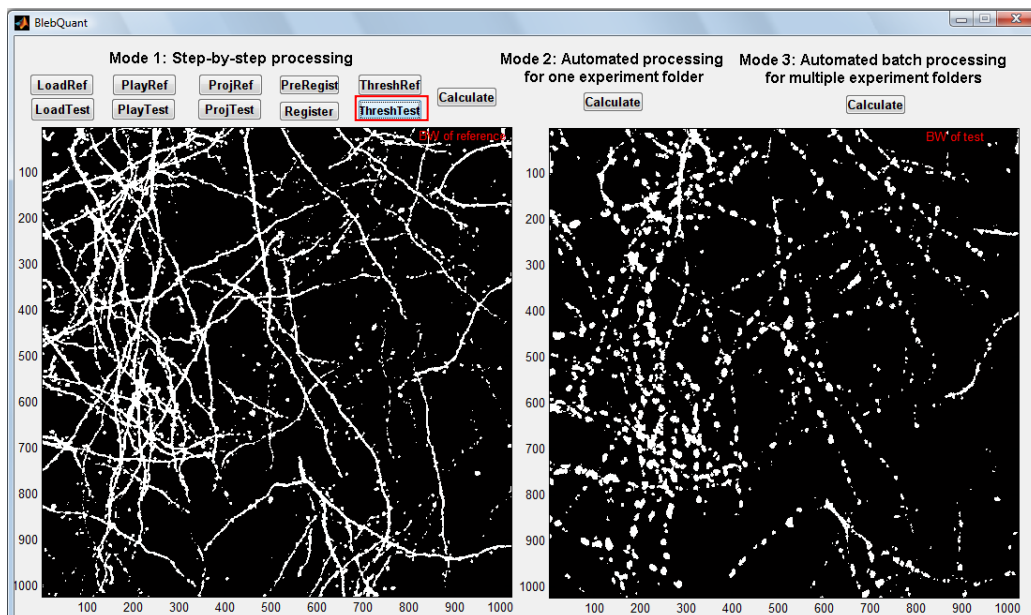


Fig. 9 Binary images following image thresholding.



	A	B	C	D	E	F	G	H	I	J	K	L	M
1	File	Percent	Mean(E)	Mean(A)	Area Th	Density	Im Size	Blocks	Blebs	Sframe	Prenum	M10mean	OTSU
2	D:\data\str_ch1.TIF	93.0855	0.742306	120.5686	376.2222	0.14204	900*900	54//55	299	1:20	80	0.148081	0.192157
3													
4													
5													
6													
7													
8													
9													
10													

Fig. 10 Example of excel results sheet.

Below is a description of the column headers in the excel results sheet. For a more detailed explanation of these terms, please refer to our paper.

“File”: filenames of processed stacks

“Percent”: calculated blebbing percentage for processed stacks

“Mean(E)”: mean eccentricity value of blebs after fine selection

“Mean(A)”: mean area value (in pixels) of blebs after fine selection

“Area Th”: upper area threshold (in pixels) for fine bleb selection

“Density”: value used to quantify dendrite density in projected reference image

“Im Size”: image size (in pixels) after image registration and cropping

“Blocks”: first number indicates blocks containing blebs in the test image and the second number indicates blocks containing intact dendrite segments in the reference image

“Blebs”: number of blebs in test image after fine selection

“Sframe”: projected frames in stack (i.e.: 1:20 denotes frames 1-20 were z-projected)

“Prenum”: number of blebs in reference image after fine selection

“M10mean”: mean intensity value of the first 10 brightest blocks in test image

“OTSU”: OTSU threshold in test image

### Step 3. Executing Mode 2 in *BlebQuant*

For a dataset where multiple image stacks desired for analysis have been saved to a folder, Mode 2 can be used to perform automated analysis. In this case, the user may be required to organize or sort and rename (if necessary) image stacks as the program is defaulted to select the first image stack as the reference image stack (i.e.: if a folder contains filenames “pre\_ch1”, “rep\_ch1”, and “str\_ch1”, “pre\_ch1” will be selected as

the reference stack and remaining stacks are assumed to be test stacks). Selecting “Calculate” under Mode 2 will allow the user to select and open image stacks from a folder (Fig. 11). As with Mode 1, results are written to an excel file (Fig. 12) which will be named and saved according to the source code in line 823 of *BlebQuant.m*.

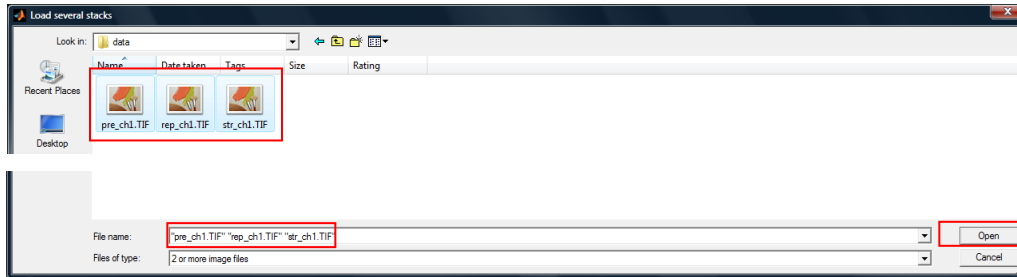


Fig. 11 Loading several stacks for processing in *BlebQuant*.

File	Percent	Mean(E)	Mean(A)	Area Th	Density	Im Size	Blocks	Blebs	Sframe	Penum	M10mean	OTSU
rep_ch1.TIF	43.45207	0.733327	81.46512	273.8456	0.14204	900*900	411/56	129	1:20	78	0.185905	0.219608
str_ch1.TIF	93.0855	0.742306	120.5686	376.2222	0.14204	900*900	541/55	299	1:20	80	0.148081	0.192157

Fig. 12 Results of blebbing analysis following Mode 2 execution.

#### Step 4. Executing Mode 3 in *BlebQuant*

Mode 3 can be used to perform automated batch processing for experimental datasets which have been saved to several different folders. Image stacks should be prepared as in Mode 2. The user will also need to prepare an excel sheet specifying pathways of all folders for analysis (e.g. “data pathway.xls”) (Fig. 13) as well as specifying in the source code on line 1120 the names of excel results sheets and the pathways to which they are to be written.

In contrast to Modes 1 and 2, selecting “Calculate” in Mode 3 will get the user to load the excel file containing specified pathways of folders for analysis (Fig. 14). This will execute batch processing and results for each data folder will be written to an excel



file. Excel files will be named and written to the pathway specified in the source code on line 1120 of BlebQuant.m.

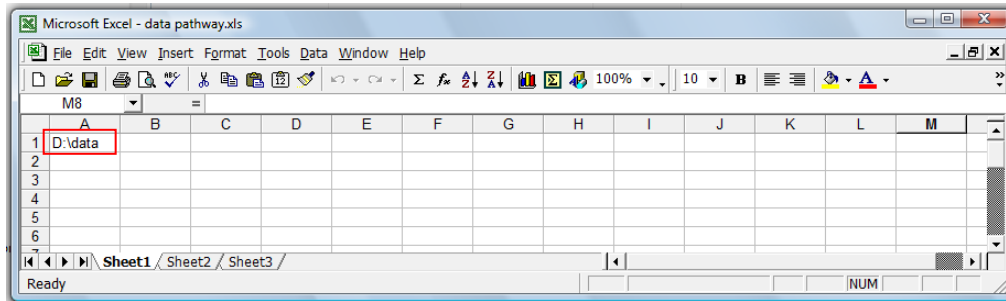


Fig. 13 Excel sheet containing pathways of experimental folders for batch processing.

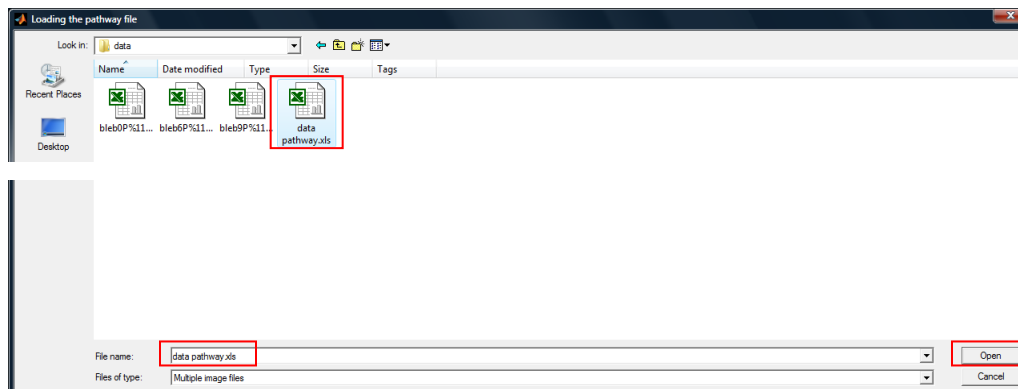


Fig. 14 Selecting excel file for Mode 3 execution.

### 3. Acknowledgements

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### 4. References

- Chen S, Tran S, Sigler A, Murphy TH. Automated and quantitative image analysis of ischemic dendritic blebbing using *in vivo* 2-photon microscopy data. *J Neurosci Methods* 2011; doi:10.1016/j.jneumeth.2010.12.018
- Guizar-Sicairos M, Thurman ST, Fienup JR. Efficient subpixel image registration algorithms. *Opt Lett* 2008; 33: 156-8.